ALGAE IDENTIFICATION AND ENUMERATION

LECTURE AND LABORATORY NOTES

COMPILED FOR THE INSTRUCTION OF

WATER WORKS OPERATORS

BIOLOGY BRANCH

DIVISION OF LABORATORIES

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PLANKTON IDENTIFICATION AND ENUMERATION COURSE

Day and Time	Subject	Section No.
Monday November 4th		
9.00 - 9.15	Welcoming Remarks	
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11.00 - 11.15	Identification of Plankton (General)	2
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1.00 - 1.30	Use of the Microscope	4
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4.00 - 4.30	General Discussion	
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9.30 - 10.00	Blue-Green Algae	6
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ALGAE AND OTHER INTERFERENCE ORGANISMS IN WATER SUPPLIES

What are Algae

Algae are plants just as trees and grass are plants. They are green, they manufacture their food in the form of starches or oils by using the energy of sunlight and the nutrients they extract from the water. In the classificiation of plants they are considered to be the most primitive group and some of the algae forms commonly found in water supplies are thought to be similar to the first life on earth. They are considered primitive because each cell is capable of carrying out the complete life history as no specialization has been developed into various tissues such as are found in the higher plants, i.e. stems, roots, leaves or seeds.

Value of Algae

The Water Works operator often looks upon algae as purely a nuisance as it clogs filters, creates nuisances and imparts tastes and odours in the water supply. Algae are, however, the basis of all life in water. On land, grass feeds the rabbit which in turn is eaten by the fox and while the fox does not eat grass, there would be no foxes if there were no grass. This is called a food chain and at the basis of the food chain in water, are algae. These plants feed minute animals that are in turn eaten by minnows which in turn provide the food for pickerel. Thus, if there were no algae there would be no pickerel. It can be demonstrated that fish production in a lake varies directly with the amount of algae that it produces and thus while it may be a disadvantage in a water supply, it is not unnatural and is a necessity for other uses we make of water. When the first men start making long distance trips into space, the food they will live off will be algae grown in the space capsule.

Size and Distribution

There are several thousand different species of algae that live in the waters of Ontario. These range in size from a plant as much as four feet tall down to cells which are so small that they can barely be seen when magnified a thousand times in a microscope. Algae live in almost any place where there is moisture and sunlight. In addition to living in the oceans, lakes and rivers down to the depth where the light can penetrate, they also live on the damp soil on the face of glaciers, and in combination with fungi to produce the lichens we are all familiar with.

Growth Requirements

Algae are very specific in their needs. The types that are characteristic of lakes are seldom found in streams and those which populate a lake in summer give way to other forms in winter. Some species can only live in very pure water and others are obligated to polluted situations and even sometimes to particular types of pollution.

As the environment controls both the numbers and species of algae it is important that these be considered in more detail. For purposes of discussion, a division can be made into the physical and chemical environments. In the physical environment, light is of prime importance. In the growth of algae, their multiplication

and manufacture of food is dependent upon the energy derived from light to drive the process. The quantity of light available for this purpose varies with the season and with the colour and turbidity of the water. It is believed that one per cent of the normal quantity of light reaching the surface is the minimum quantity necessary for growth. A second important factor is temperature; various species of algae grow best within a narrow range of temperature, thus, one group of algae may dominate the summer populations, while others will grow actively during cold water periods.

The chemical environment is the second important factor controlling the number and species of algae. Algae in the water are like plants on land in that they respond to the general fertility. Where there is an abundance of nutrients, providing all the food that is required, an abundance of plants might be expected, whether it is on land or in the water. In water, nitrogen and phosphorus supplies are generally the most limiting, particularly in our southern waters. Northern waters tend to be poor in many nutrients and seldom develop high algae populations.

Most of the waters where algae problems exist are waters that are rich in nutrients and produce continuing dense populations of algae.

Algae Problems in the Operation of Water Supplies

The operator can be faced with problems created by algae of several kinds. In all cases, they result from an over-abundance but the numbers required to create this difficulty will vary.

Filter Clogging

The reduction of filter runs, caused by the coating of the surface of the filters with large numbers of these minute plants is probably the most common and serious problem that algae create for the Water Works operator. Certain waters at certain times of the year produce a great abundance of the filter clogging species and under the worst conditions may reduce the production of water through a filter to a point where there is hardly sufficient to back wash it. The lake diatoms are the most common trouble maker in this regard but certain of the summer blue-green algae will also develop in sufficient numbers to reduce filter runs.

The obvious way to solve the problem of short filter runs is to remove the algae before they get to the filter. The common method of removing algae from raw water is the use of settling basins which may include flocculation and pre-chlorination. Microstrainers are also being used to remove the algae before the water is filtered. A third method is to apply algicides to the raw water and thus remove them before the water enters the plant.

The principle of removing algae by flocculation and sedimentation involves trapping the algae cell in the alum floc and carrying it to the bottom with other unwanted solids from the water. When algae populations are very high they often hold the floc in suspension long enough for it to pass through the settling basins and onto the filters. This floc and the algae can be settled if weight can be added to the floc. A slurry of ordinary clay mixed and fed during the periods of difficult times will do much to get the operator over a short term period of difficulty. Increased dosages of alum and heavier pre-chlorination will also assist in alleviating short filter runs.

Where short filter runs create a chronic problem or where the capacity of a plant is limited by high algae populations at certain times of the year, microstraining can be used to remove most of the algae before the water reaches the filter. This and chemical treatment will be discussed more fully in this talk.

Algae and Tastes and Odours

Algae are capable of producing tastes and odours that will persist through treatment and cause consumer complaints.

There is much published information on taste and odour problems caused by algae. Different algae have been shown to cause different flavours and odours that have been variously described as pigpen, grassy, musty, cucumber, etc. While there is no doubt that these problems do occur it has been observed that in this province most of the difficulty does not come from algae but is the result of the decomposition products of the rooted aquatic weeds. Raw water supplies that come from shallow weedy lakes almost invariably have a continuous or intermittent problem with tastes and odours. This problem is usually most acute late in the fall when the ice cover is first formed and again in the spring at the time of the break-up of ice cover.

Two methods are commonly used in controlling tastes and odours: one, the feeding of activated carbon and two, variations in the method of chlorination. Activated carbon is the only sure method of taste removal, but this on a continuous basis is somewhat expensive. The use of chlorine dioxide or chloramine at various points of application in the plant may assist in controlling tastes and odours but this is an individual problem with each Water Works and can only be determined through experimentation.

One of the common sources of tastes and odours to a water treatment plant is the decomposition of accumulations in the settling basin. A number of instances have been observed where water leaving the treatment plant was in poorer condition than in the raw water coming in. A short term routine in cleaning settling basins should be practiced by all Water Works in order that the decomposition products from the accumulated solids do not impair the quality of the water.

Growths in Reservoirs

A third common problem that algae create in Water Works operation is the growths in reservoirs. Here the algae may grow attached to the walls where they form a heavy mass of material alive with crustaceans and insect larvae. Or it may be the free floating type similar to that removed at the filtration plant. Either one may impart tastes to the water and the odd little animal that comes through the taps, may shake the confidence of the consumer in the purity of the supply. Probably the most common algae causing difficulty in reservoirs is one of the large species called Chara. This algae grows to a height of two or three feet in a soft mud bottom and is typical of the cold hard water commonly found in spring water sources. Where such water is collected and stored in an open reservoir this algae invariably grows and is difficult to control.

Control of Algae

There are two basic means of controlling algae and solving the problemsthat they create: one, by controlling the environment in such a way as to make it an unsuitable place for them to live, and two, by killing them with chemicals. The latter method is less satisfactory in the long run as it necessitates adding chemicals that are costly on either a continuous or intermittent basis. Controlling the environment is a satisfactory means though this is not always possible.

Environmental Control

The exclusion of light is probably the most common environmental control. The easiest method is simply to cover the reservoir although this is often not done and algae problems continue year after year. A second method of reducing light is to use activated carbon to induce an artificial turbidity. While this is only a temporary measure and must be repeated every few days it has the added advantage of absorbing taste and odours from the water while in suspension and keeping the bottom accumulations sweet. Carbon can only be used in the raw water where the treatment following includes good sand filtration. Another method of excluding the light would be covering the reservoir with black plastic. While this has not been used it would be effective in excluding light and be relatively inexpensive and easy to apply.

The regulation of algae growth by controlling the nutrients is not always possible though effective where it can be applied. In choosing a new supply care should be taken to utilize water of low fertility as judged by chemical analysis and the algae population that it maintains. In this case a limnologist should make several inspections at various times of the year to determine the numbers and kinds of algae present and to assess the suitability of the water. Where the municipality controls the land adjacent to the supply, care should be taken to keep out surface drainage and other possible nutrient sources. Run-off from farm buildings, domestic sewage and certain industrial wastes are rich in plant nutrients and should be avoided as only small amounts of these fertilizing substances can induce the development of high algae populations.

Chemical Control

While there are many algicides sold today, only two are suitable for use in domestic water supply, namely copper sulphate and chlorine. The cheapness and availability of copper sulphate and its safety from the public health standpoint makes it the most satisfactory chemical to use. The effectiveness of copper sulphate varies somewhat with the chemical composition of the water. In hard water the copper precipitates rapidly and thus more is required than in soft water. As a rule of thumb, .15 ppm. will kill most organisms in surface waters. Copper sulphate is very toxic to fish and 0.5 ppm. is about all they will stand. Chlorine is sometimes used as an algicide especially where water is taken into a holding basin. A residual of 1 ppm. will kill most algae forms. While chlorine is more expensive than copper its use is understood by operators and equipment is usually available for feeding it.

In applying chlorine, a rough calculation must be made of the volume of water being treated and the pounds of chlorine required to satisfy the demand and still provide a residual of 1 ppm. The calculation is used as an initial guide, then followed by chlorine tests to provide the final adjustment. A similar calculation must be made for determining the amount of copper sulphate but more care must be exercised as no

simple test can be used as a guide. To do this, the surface area of the water to be treated must be obtained together with the average depth of the water. When multiplied these two figures give the volume of water in cubic feet. The total number of pounds of water may then be calculated by multiplying the volume by 62.3. As one part per million (ppm.) equals 1 pound per million gallons, the treatment of a reservoir with 0.5 ppm. would require one half a pound for each million pounds of water.

Area X Average depth X 62.3 - lbs. of water in reservoir

1 ppm. -1 lb per million lbs. of water

Copper sulphate is sold in a variety of crystal sizes. The method of application varies with the grade of crystal used. In general, the finest crystals may be distributed on the surface of the water as they will immediately dissolve. Larger sized crystals may be dissolved in water and pumped as a spray or they may be put in a burlap bag and towed through the water in such a way as to provide an even distribution of the calculated amount of chemical over the entire surface of the reservoir. The operator must know the depth of water as he applies the chemical and see that the deeper water receives proportionally more chemical than the shallow areas.

The ideal time to apply chemical is when the algae population is rising but before the condition becomes acute. If treatment is postponed until a very dense growth of algae occurs the sudden killing of this material and the subsequent decomposition may remove all the oxygen from the water causing it to go septic, kill the fish, and become foul tasting. If the condition gets out of hand before treatment can be applied half the reservoir should be treated first, to reduce the population, and after a week or so has been allowed for this material to decompose the total reservoir area can then be treated.

Microstraining

Microstraining as a method of water treatment has been introduced into Ontario within the past five years, and there are now five installations operating on municipal water supplies. The development of this means of filtration was made possible by the invention of an extremely fine wire mesh capable of removing such small particles as algae from the water and yet capable of passing high volumes of water. The principle of the microstrainer is simply a rotary screen where the raw water is fed to the inside and flows out through the screen material. The drum is about three quarters immersed and as it turns around, a jet of water played on the surface of the screen knocks down the accumulated solids into a hopper and from there are carried to waste.

In water treament the microstrainer has two uses: (1) as pretreatment for algae removal ahead of conventional sand filters, (2) as sole treatment for waters for the removal of algae and other extraneous material where turbidity is not a problem. It has been our experience that they have proven very effective in extending the operating time of conventional sand filters during times of heavy algae bloom. In one instance, runs of not less than 20 hours have been obtained where previously 6-hour runs in summer were not uncommon and as little as two hours were experienced. Where this equipment is used as a sole means of water treatment it should never be installed with the thought of reducing turbidity. Where it has been ued solely for the removal of algae and the protection against the variety of water fleas, insect larvae, leeches and aquatic worms, that commonly pass through unprotected water supplies, it has been found to be very satisfactory.

Operation

Some of the microstrainers installed in the province have been set up on an automatic control system and some are operated manually. The system used will depend on the individual plant. In general, they are easy to operate and require only the normal lubrication and an occasional wash down. Over a period of time some permanent plugging of the screens will take place that is not backwashed by the water jet. When this occurs, the strainer must be drawn down and a 12% sodium hypochlorite solution applied directly to the fabric while the screen turns over slowly. It should be emphasized that concentrated chlorine solutions from a chlorinator or from chlorine powders are not effective in rehabilitating the screen capacity.

The reason for the sliming of the fabric is not well understood. The time between washings has varied anywhere between one day and six months and in one or two instances difficulties due to lessening of filter capacity over a period of one or two days has occured. In all cases, the screens have been quickly rehabilitated with the hypochlorite wash and an investigation is now underway to botain a continuous method of protection against this short term loss of capacity.

Water Main Infestations

While water main infestations do not necessarily come under the title of this paper, there has been considerable interest in this matter in the past year, and so perhaps a little of the fact and fiction should be separated.

It is probable that most if not all water distribution systems contain living organisms of some kind. A brief survey of the literature indicates the wide variety of animals that have caused difficulty from time to time. These have included nematodes, aquatic earth worms, snails, clams, a variety of aquatic insect larvae, leeches, Gordian or horse hair worms, Daphnia or water fleas, etc. Many of these have occured in municipal supplies having complete treatment with flocculation and sand filtration. The method of entering the distribution system often remains a mystery though in many cases it is thought that the minute egg pases through the sand bed and develops subsequently in the distribution system. This problem is not more wide-spread because the inside water main is relatively clean so that food is available only in very limited quantities. In some cases the life cycle cannot be completed entirely under water so that insects such as blood worms cannot reproduce in the water main.

Nematodes

Earlier this year an article on worms in the drinking water in a leading U.S. News magazine aroused the public and many inquiries were directed towards this organization and undoubtedly the municipalities.

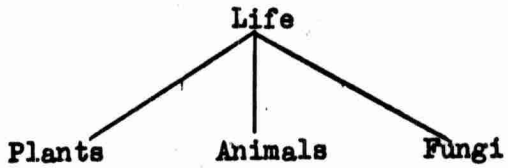
The worms referred to were round worms or Nematodes. These are a very small animal barely visible to the human eye. Some authorities consider them to be the most numerous animals on earth as they are found in the soil on lake bottoms, along the shores, in sewage sludge and in fact in almost any sample of earth. In view of their great numbers, it is not surprising that a few of them find their way into water distribution systems. Here they are able to subsist on the thin coating of slimes lining the water main and in organic decompositions in low flow sections of the distribution systems.

In this country, Nematodes have no public health significance, they carry no diseases, and are not human parasites. Although it is not likely known, many of the Nematodes are consumed by an individual every year in raw fruits, salads, vinegar, and perhaps even in his drinking water. They are quite resistent to chlorination and the levels normally applied for the control of pathogenic bacteria are not sufficient to kill the nematodes. In this respect, we are fortunate as this climate controls some species that are a serious human problem in the middle east and Asia.

Control of Infestations

As most of the organisms that inhabit water mains are resistent to normal Water Works sterilization procedures and as no chemicals are suitable for adding to water to control this type of nuisance organism, good housekeeping is the only effective control. As much as possible of the organic material should be kept from the water mains. This is best accomplished through chemical precipitation and sand filtration. In laying water mains low flow areas should be eliminated as much as possible, as these provide a refuge, and where dead ends occur they should be flushed routinely. In this way, it will be possible to maintain the confidence of the consumer in the products you deliver.

IDENTIFICATION OF PLANKTON (GENERAL)



Plants

Plants are photosynthetic, i.e. in the presence of sunlight they can synthesize inorganic nutritional materials to provide for growth and reproduction. This is made possible by the presence of the pigment chlorophyll which imparts the green colour to all plants, large and small. While the proper classification of some of the single-celled forms of life is open to dispute among mocrobiologists because some have both plant-like and animal-like characteristics, a simple means of differentiation is to categorize all organisms which contain chlorophyll as plants. Sometimes other pigments are present in algae which impart a blue-green or brown colour to certain forms of these tiny plants because the chlorophyll is masked by these other pigments. (e.g. blue-green algae - phycocyanin).

PHOTOSYNTHESIS

$$\text{Carbon Dioxide} + \text{Water} \xrightarrow[\text{and chlorophyll}]{\text{in the presence of sunlight}} \text{Starches \& Sugars} + \text{Oxygen}$$

This is an extremely simplified formula indicating what takes place as plants manufacture their food. A balanced condition is provided by the fact that animals breathe the oxygen produced by plants in order to metabolize their food and release energy for movement and other bodily activities, at the same time producing carbon dioxide which is essential to the plants.

Fungi

Fungi are a somewhat specialized group which for a long time have been classed by most biologists as plants. However, they are distinctly different in that they do not possess chlorophyll and are unable to synthesize their own food. Fungi are able to secrete enzymes which change insoluble food to a soluble form which is assimilated and metabolized within the cells.

Animals

Most animals ingest and break down solid food of organic origin although there are some unicellular forms which assimilate soluble food materials through their cell membranes. They cannot produce their own food and never contain chlorophyll.

Planktonic forms of interest to us represent the Plant and Animal Kingdoms. Algae are the tiny plants, mostly microscopic in size, that are extremely influential in affecting water supplies in certain areas. In addition, there are both unicellular and multicellular animals which are encountered and which may cause nuisances also.

Algae

These tiny, chlorophyll-bearing plants may be single-celled or cells may be grouped together in filaments or colonies.

Although there are quite a number of major groups of algae which are recognized by taxonomists, for purposes of simplification, the algae which are significant in water supplies may be grouped into four different types. These are as follows:

1. Blue-green algae
2. Non-motile green algae
3. Pigmented flagellates
4. Diatoms.

Naming Planktonic Organisms

A complex system of classification has been worked out in which all forms of plant and animal life occupy a position. Organisms which are exactly like other organisms in all respects are placed together in groups called species. Individuals of many different species of plankton resemble each other so closely and are so difficult to differentiate that they are identified only to a particular genus. The genus (plural genera) is a much larger group containing the many species which have similar characteristics. We will be identifying only to genera.

ANIMAL LIFE ENCOUNTERED IN WATER SUPPLIES

Unicellular forms of animal life are collectively referred to as Protozoa. Five major groups of Protozoa are recognized as follows:

1. Sarcodina - e.g. Amoeba, Arcella
2. Mastigophora - e.g. Bodo
3. Ciliata - e.g. Paramecium, Vorticella
4. Sporozoa - not of interest - occur as parasites in plants and animals
5. Suctoria - e.g. Actinosphaerium.

Representatives of the Sarcodina groups have pseudopodia, finger-like processes which develop and are constantly changing in shape as the animal literally "flows" as it moves along very slowly. Pseudopodia may also be extended to engulf food particles which are assimilated through the cell membrane of the animal into the protoplasm within the cell. Protoplasm is the jelly-like constituent of all plant and animal cells in which all of the basic life processes occur. The Mastigophora group is comprised of those forms which bear flagella (singular - flagellum). These are whip-like appendages which are used in movement. Flagellates move in corkscrew-like fashion. The possession of cilia is the outstanding characteristic of the Ciliata. Cilia are hair-like appendages which cover the body of the cell or are located at the anterior end of the animal. They are used in movement and in some cases for capturing food. Both free-swimming (Paramecium) and attached (Vorticella) forms of ciliates are often encountered. The Suctoria possess tentacles which are used to sting other organisms which pass nearby and to suck the contents from their victims.

Multicellular Animals

Rotifers

These more complex animals have one or two crowns of cilia at the anterior end of the animal which resemble wheels. These cilia beat to permit movement and to create suction currents for drawing in food particles. The mastax is a powerful set of jaws which is clearly evident in the body of the animal which grinds up food with a hammer-like action.

Nematodes or Round Worms

Small aquatic ~~nematodes or~~ "sludgeworms" are related to terrestrial earthworms. These tiny worms are able to withstand low dissolved oxygen conditions and are frequently observed as pink or red carpets on the bottoms of polluted streams. They are frequently observed on the filter beds in water filtration plants.

Midge Larvae or Blood Worms

These segmented insect larvae are often vivid red in colour and are wormlike in character. They may be distinguished from the aquatic nematodes by the fact that they are somewhat thicker in diameter and microscopic examination may reveal hooks and breathing tubes.

Daphnia and Cyclops

These are tiny relatives of the common crayfish which feed on algae and are an important food item for small fishes. They appear as tiny white specks which move through the water with a jerky motion.

Scuds or Amphipods

These are somewhat larger crustaceans than the Daphnia and Cyclops, which may grow up to half an inch in length. They move smoothly through the water and their abdominal gills may be seen functioning when the animal is at rest.

Hydras

These tiny fresh-water coelenterates have a columnar body usually 15 to 20 mm. in length with a ring of tentacles around one end of the body, which are used to paralyze and capture prey. Hydras have been known to live on the walls of filter beds and to sometimes clog filters when they reach particularly high numbers in lake waters.

Moss Animals or Bryozoans

Various treatment plants along Lake Ontario and Lake Erie have reported the presence of free-floating statoblasts of Pectinatella. The statoblasts are reproductive bodies from which larger jelly-like colonies develop. Other bryozoans form mosslike brownish mats which are attached to the bottoms of lakes or streams.

Terminology Used in the Description of Protozoa

Pellicle - the cell membrane which determines the shape of the animal and encloses the inner components of the cell.

Protoplasm - the jelly-like substance which is contained by the pellicle in which the animal's life processes function. The protoplasm is divided into a clear outer portion called the ectoplasm, and a granular inner mass, the endoplasm.

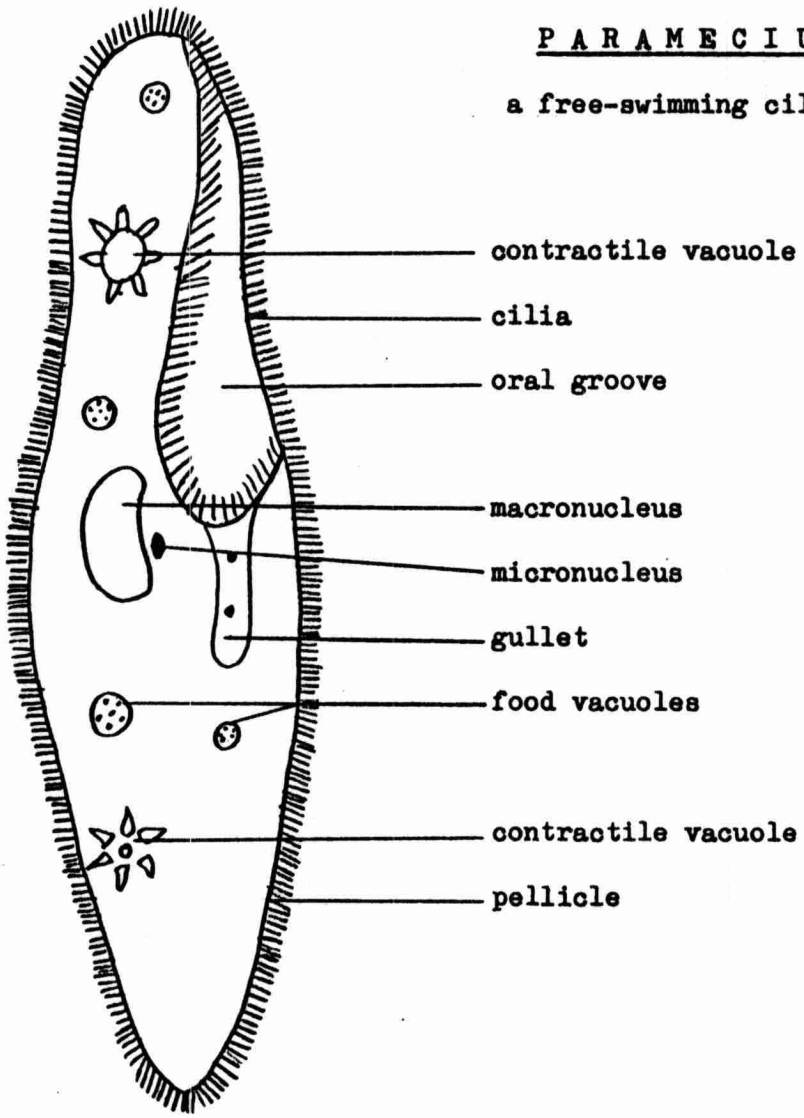
Macronucleus - the larger nucleus which governs bodily activities within the cell. Not present in Sarcodina.

Micronucleus - the smaller nucleus which is involved in reproduction.

Contractile Vacuole - a relatively large, clear structure which is responsible for gathering and excreting water from the cell.

Food Vacuoles - structures in which food is being broken down by enzymic action.

Oral Groove - present in ciliates - opening lined with cilia into which food particles are drawn.



LABORATORY - FAMILIARIZATION WITH MICROSCOPE AND LEARNING TO RECOGNIZE ANIMAL PLANKTON

Objectives

1. For beginners to learn how to use microscope.
2. To learn to identify animal life encountered in plankton counting and to associate these with basic animal types.

A. Use of the Microscope

1. Note the major parts - objectives, eyepiece, stage, light source, coarse adjustment knob, fine adjustment knob, co-axial handle to position slide.
2. The bottom left-hand corner of the Sedgwick-Rafter cell is centered under the objective. Note how the image appears to the eye - upside down, and on the opposite side.
3. Practice focusing with the objective fixed over the edge of the Sedgwick-Rafter cell to gain an idea of the depth of field at 100X.
4. The magnification achieved is the product of the power of the eyepiece and of the objective. A 10X eyepiece and a 20X objective provides a magnification of 200X.
5. Practice moving the slide on the stage using the co-axial handle - forward and back, from left to right and vice versa.
6. Prepare a "wet mount" using a Daphnia from the collection of living materials and study it at 100X. Make a sketch of the organism on the blank sheet provided at the back of the manual for this purpose.

Identifying Animal Plankton - See next page.

Study and sketch as many of the living specimens as time will permit. Identify these using reference books available and with the help of the instructors. Methocel (methyl cellulose) is available for you which will slow the organisms down so that they may be studied more easily. Make a tiny ring on the slide using the methocel and place a drop of the sample containing the specimens inside the ring.

Identification of Animal Plankton

Protozoa - Single-celled or aggregate of similar cells combined in a colony. Have flagella, cilia or pseudopodia.

Rotifers - Two wheel-like crowns of cilia at anterior end. Can be either free-swimming or attached. Mastax or grinding jaws can be seen working inside the body of the animal. Multicellular.

Microcrustaceans - Daphnia

- single "eye" very apparent
- one pair of branched antennae which are used for locomotion
- animal enclosed in shell (carapace).

Copepods

- moves by means of two large unbranched antennae
- egg packets sometimes visible
- abdomen ends in two small projections.

Amphipods

- also Crustaceans
- have flattened leaflike swimming and breathing appendages.

Nematodes - round, slender worm-like forms - sinuous movements.

Midge largae

- no jointed legs present in larval stage
- look for hooks, breathing tubes.

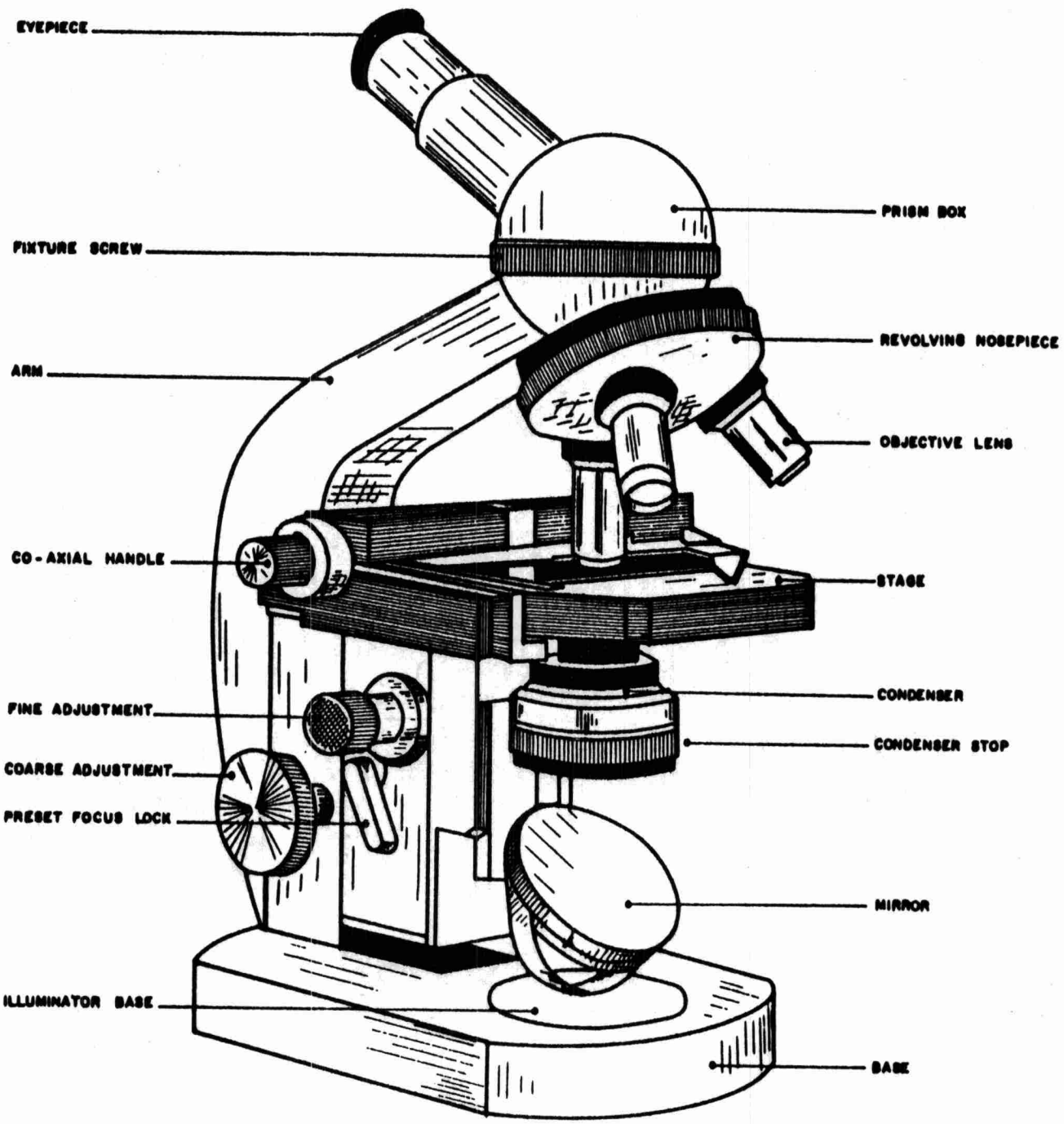
Hydras

- have rings of tentacles
- reproduce by "budding".

Bryozoans - reproductive statoblasts are round with borders of anchor-like hooks.

Plankton - free-floating forms of animal + vegetable organisms

PARTS OF THE MICROSCOPE



Blue Green Algae

- single cells
- lack of differentiation
- chlorophyll uniformly distributed throughout the cell
- no nuclear membrane
- no flagella
- spores often present
- endospores + heterocysts akinetes
- some have gelatinous sheath
- single, celled, colonial, filamentous
- thin membrane

Non-Motile Green Algae

- more complex
- chlorophyll in chloroplasts
- nucleus
- no flagella
- no spores
- gelatinous matrix
- single, celled colonial, filamentous
- thick cell wall

Pigmented Flagellates

more complex

chloroplasts present

- nucleus
- flagella
- no spores

gelatinous matrix

- non-filamentous
- single celled, colonial
- cell membrane

Diatoms

complex

plastids - other pigments

- amber, orange, brown
- chlorophyll + chromatin (brown)
- nucleus
- no flagella
- no spores
- usually no gelatinous matrix
- single celled, colonial, filamentous
- rigid cell wall of silica

CHARACTERISTICS OF MAJOR TYPES OF ALGAE

Algae, which are important in water supplies, may be placed in four general groups - the blue-green algae, the green algae, the diatoms and the pigmented flagellates.

Blue-green Algae

Blue-green algae are most prevalent in late summer, following lengthy periods of warm weather. They are the most simple form of algae and are perhaps best described by what they do not have. Characteristics are as follows:

(1) no chloroplasts, or definite bodies containing chlorophyll, are present - chlorophyll is dissolved throughout the cell.

(2) no nuclear membrane is apparent.

(3) they have no flagella (whip-like appendages to allow movement).

(4) some species have specialized spores called akinetes, endospores and heterocysts. These spores carry the algae through periods when unfavourable environmental conditions are encountered.

(5) many species have a definite gelatinous sheath or envelope.

(6) there are unicellular, colonial and filamentous species.

Examples - Anabaena (filamentous), Anacystis (colonial), Oscillatoria (filamentous), Chroococcus (single-celled or colonial).

Non-Motile Green Algae

(1) have chloroplasts - chlorophyll is present in definite bodies.

(2) have a semi-rigid cell wall.

(3) have a well-defined nucleus.

(4) have no flagella or other locomotor appendages.

(5) there are filamentous, colonial and single-celled species.

Examples - Spirogyra (filamentous), Scenedesmus (colonial), Oedogonium (filamentous), Closterium (single-celled).

Pigmented Flagellates

(1) have flagella for movement - one or more per cell.

(2) a thin cell membrane is usually present.

(3) have a well-defined nucleus.

Non-Motile Green Algae

1.) Filamentous - branched + unbranched

2.) Coccoid - independent, free-floating units of plankton,

1.) Cladophora - filamentous, tapering, attenuation ⊢ - holdfasts

Stigeoclonium - gradual tapering

- chloroplast shape + position

Spyrogyra - pyrenoids - starch formation

Ulothrix - parietal chloroplast

Nougeotia - axial chloroplast

- reproduce by 1.) fragmentation
2.) + cell division
3.) + gametes

2.) Coccoid - contain starch -

- coenobe - colonial form - increase in size of cells, but not in number - Scenedesmus

- variety of sizes + shapes.

- basis for food-supply for other animal life

Micractinium

Spyrogyra

Stigeoclonium - tapering, branched

Rhizoclonium - few branches - reduced + colourless

Nougeotia - axial chloroplast

Ulothrix - parietal chloroplast

(4) a light-sensitive eyespot is usually present.

(5) contain chloroplasts in which chlorophyll and other pigments are present.

(6) may be single-celled or colonial.

Examples - Euglena (single-celled), Synura (colonial), Chlamydomonas (single-celled), Volvox (colonial).

Diatoms

(1) have a very rigid cell wall making up a two-part shell composed of silica (glass) - this shell has regular lines of fine dots or markings.

(2) pigments give the cell a brownish colour - pigments are contained in definite bodies called plastids.

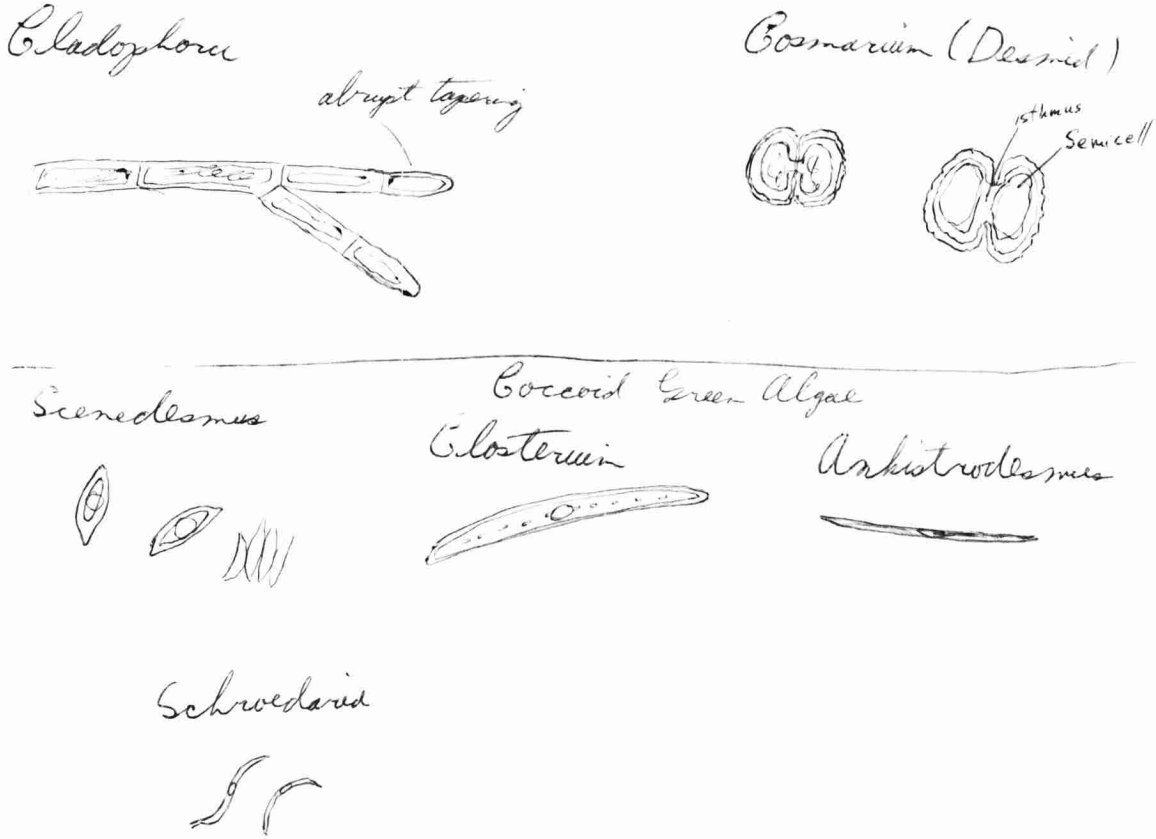
(3) a nucleus is present.

(4) they may be circular or elongated in shape.

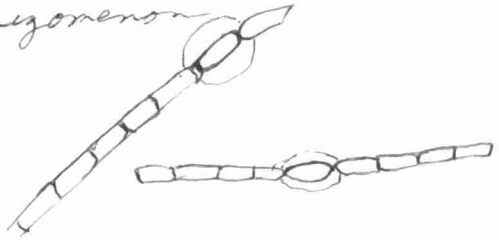
(5) some are capable of slow movement.

(6) may be single-celled, colonial or filamentous.

Examples - Navicula (single-celled), Melosira (filamentous), Fragilaria (colonial), Cyclotella (single-celled).

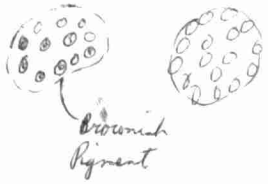


Aphanizomenon



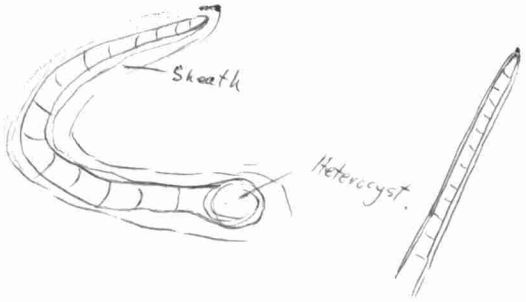
Tolypothrix

Gomphosphaeria



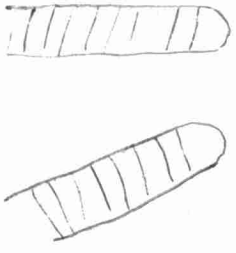
Agmenellum

Rivularia

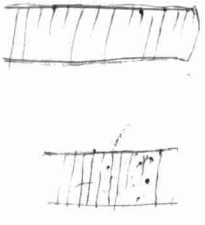


Anacystes

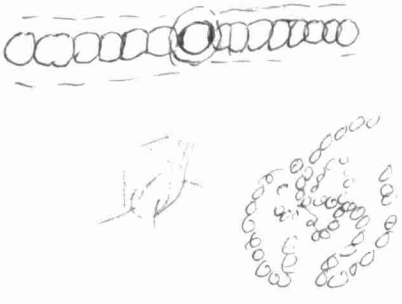
Oscillatoria



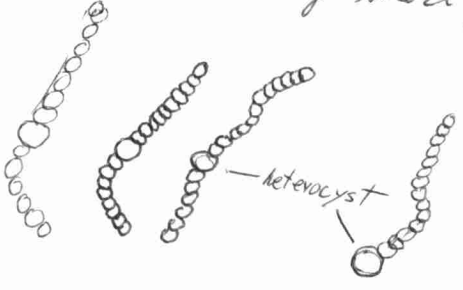
Lyngbya



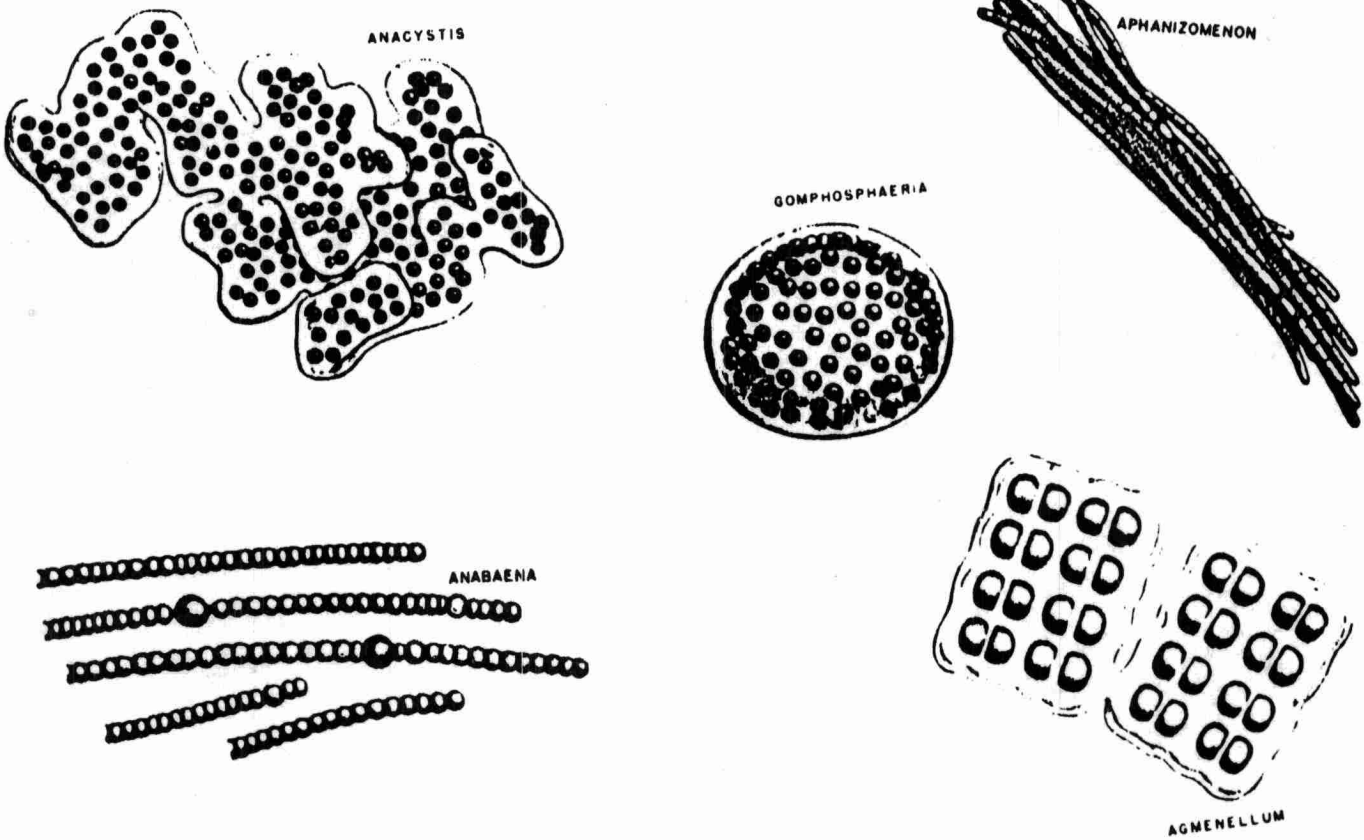
Anabaena
sclum



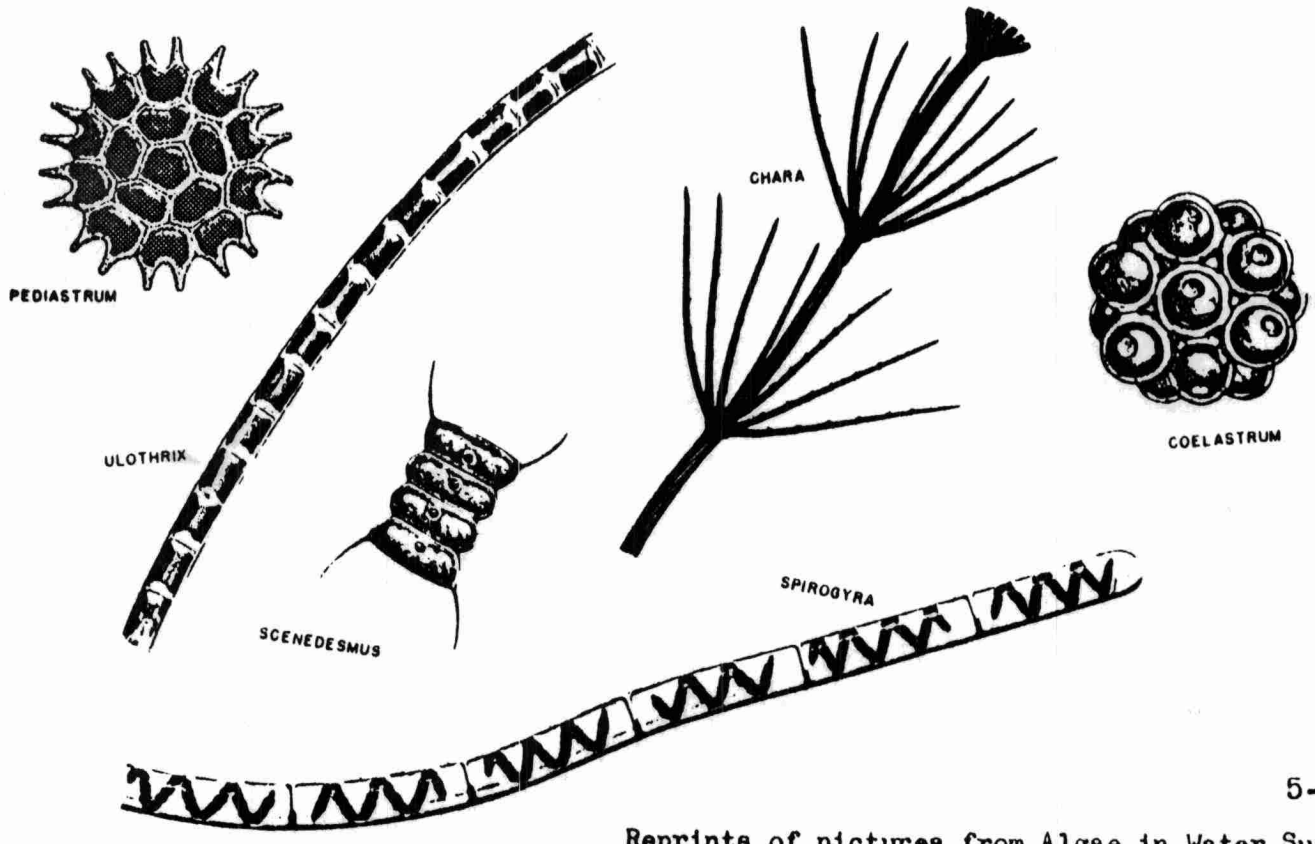
Nostoc
- grape-like clusters
- gelatinous mass of short filaments



BLUE GREEN ALGAE



NON-MOTILE GREEN ALGAE (Including Filamentous)

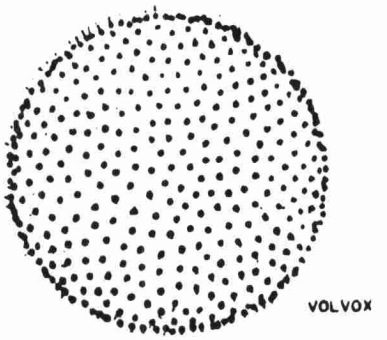


Reprints of pictures from Algae in Water Supplies
U.S. Department of Health, Education and Welfare

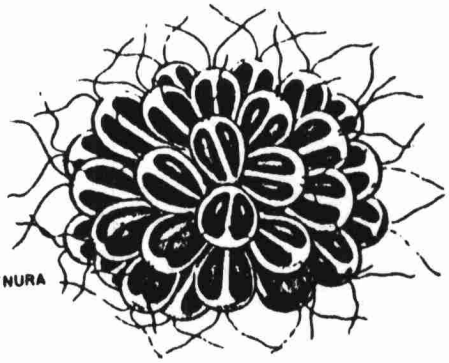
Dinoflagellates
- tough, thick outer wall

Armored Flagellates

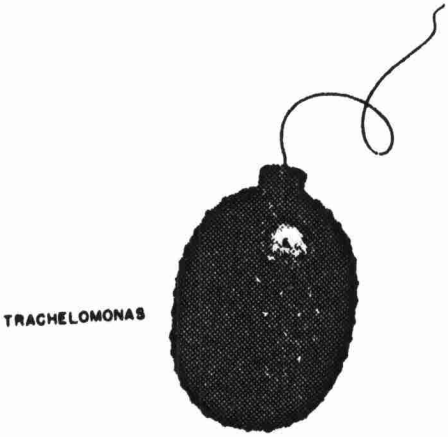
PIGMENTED FLAGELLATES



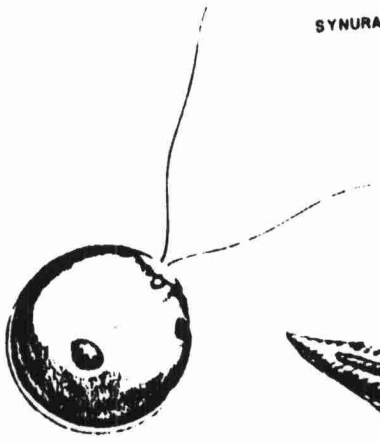
VOLVOX



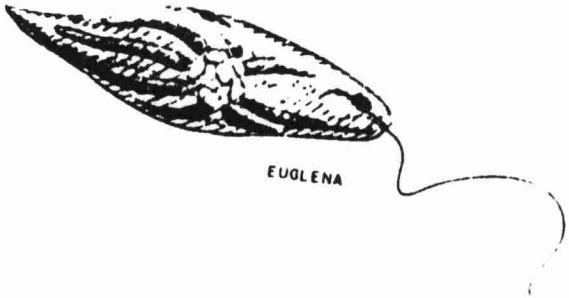
SYNURA



TRACHELOMONAS



CHLAMYDOMONAS

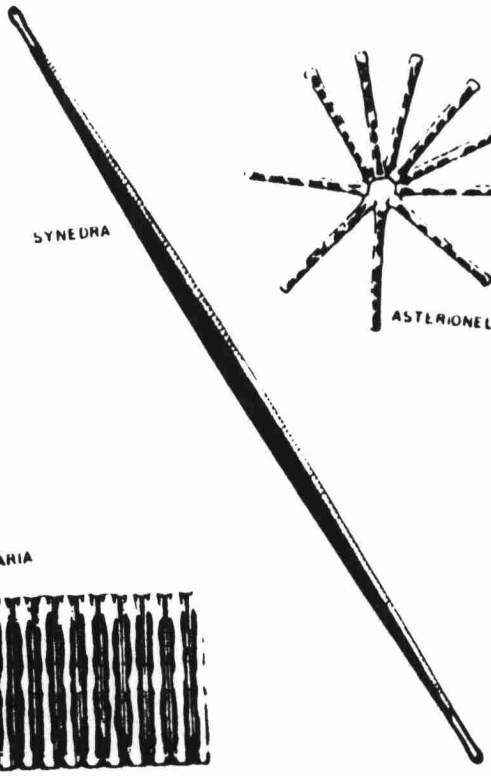


EUGLENA

DIATOMS

MELOSIRA

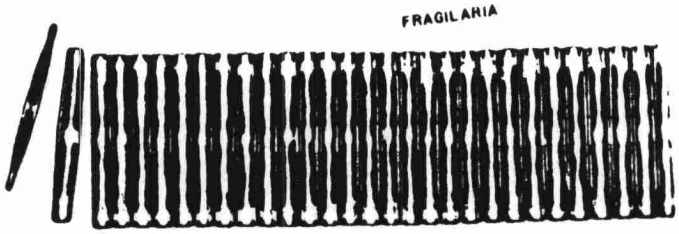
NAVICULA



SYNEDRA

ASTERIONELLA

TABELLARIA



FRAGILARIA

Reprints of pictures from Algae in Water Supplies
U.S. Department of Health, Education and Welfare

- reproduce by fission or fragmentation

Oscillatoria - homogone

Chroococcus

glycogen
glycoprotein
pseudovacuolated
- phycocyanin

Trichome - string of cells inside a sheath

false branching - two or more trichomes in a sheath

akinetes - sausage or oval-shaped

heterocysts

endospores

BLUE-GREEN ALGAE

Characteristics

1. The blue-green algae have a simpler structure than any of the other algae we are to study, and they are considered to be only slightly more complex than the bacteria.

2. The presence of the blue pigment, phycocyanin, produces the blue-green colour which is characteristic of members of this group. Chlorophyll is present in addition to phycocyanin and is distributed throughout the cell rather than being contained in chloroplasts.

3. There are no organized nuclei in the cells of blue-green algae, no vacuoles, flagella or other locomotor appendages.

4. Many species have a pronounced gelatinous sheath which may surround filaments or, in colonial species, the cells may be imbedded in a gelatinous matrix.

5. Specialized cells are present in some species, such as akinetes, endospores and heterocysts. Akinetes and endospores may carry the algae through periods when unfavourable conditions are encountered. New filaments may sometimes be formed from heterocysts in filamentous species such as Anabaena and Aphanizomenon.

6. These algae reproduce by asexual means such as fission (simple cell division) and the filamentous forms by fragmentation. In certain forms such as Oscillatoria, specialized separation discs may form in a portion of the filament and the section between these two discs, called a hormogone, will break away to form a new filament.

7. Single-celled, colonial and filamentous forms are all to be found.

Significance

When a "bloom" of blue-green algae develops, the algae sometimes drifts into bays or along beaches where it decomposes. As decomposition takes place, the mass of algae becomes unsightly, and creates foul odours and even toxins for some species. The decomposing algae are buoyed up to the surface of the water because "pseudovacuoles" or pockets of gas develop. There is usually a change to a yellowish colour as the algae degrades.

Blue-green algae are also able to clog filters and intake screens.

Examples of Blue-green Algae

1. Anacystis - includes Coelosphaerium, Gloeocapsa and Chrococcus.

   - free floating colonies
   - great variety in size and shape of colonies
   - frequently pseudovacuolated, floating at top of cell.

2. Anabaena - filamentous

- may be single filaments or groups of filaments
- trichomes often form irregularly twisted loops
- heterocysts are present which are larger than the vegetative cells and clear in colour
- akinetes tend to be sausage-shaped.

3. Aphanizomenon - filamentous form

- cells smaller than in Anabaena and trichomes are straight with cylindrical akinetes in evidence
- often trichomes are held together and resemble tiny bundles of wheat
- heterocysts never occur at the end of filaments.

4. Oscillatoria

- moves with slow oscillating movements when living
- has no akinetes or heterocysts - hormogonia are formed and break away to reproduce by fragmentation
- no visible sheath.

5. Lyngbya

- similar to Oscillatoria, but has a pronounced yellowish sheath

6. Agmenellum

- cells are arranged in flat plates in a gelatinous envelope.

7. Gloeotrichia

- have radially arranged filaments which taper distally
- tiny gelatinous balls.

LABORATORY: THE IDENTIFICATION OF BLUE-GREEN ALGAE

1. Using the key in "Algae in Water Supplies" endeavour to determine the genus to which the sample of algae provided by the instructor belongs. Even if you know the algae, follow through the key for practice.

2. Examine the preserved materials to become familiar with the genera available and terminology related to classification. Make sketches for future reference.

3. Examine Aphanizomenon, Anabaena and Anacystis under both low and medium power. Note the heterocysts and akinetes in Aphanizomenon and Anabaena.

NON-MOTILE GREEN ALGAE - FILAMENTOUS AND COCCOID

A. Filamentous

Characteristics

1. These algae are composed of cells held together end to end in filaments which may either be attached or free-floating. In some green algae the filaments are branched and in others they are not. Gelatinous envelopes are present in some species. Filaments may or may not taper towards the tips.

2. Each cell of the green algae contains an organization centre called a nucleus which is seldom readily apparent.

3. A chloroplast (or chloroplasts) is the most outstanding structure present and this contains the pigment chlorophyll which is essential for food production. The chloroplasts vary in size, number and form in different algae. Some are "parietal" i.e. they lie close to the outer cell wall; others are "axial" i.e. they extend through the central axis of the cell. Some chloroplasts, as in Spirogyra, are very distinctive and render identification possible on this feature alone.

4. Reproduction may occur by cell division, fragmentation or by the formation of specialized spores. Sexual reproduction, involving the production of reproductive cells called gametes, is not uncommon.

Significance of Filamentous Green Algae

1. They may promote development of animal plankton in storage reservoirs - provide proper conditions for growth of Daphnia etc.

2. They may clog filters and intake screens at water treatment plants.

3. Foul odours may develop where these algae are washed ashore, e.g. Cladophora problem - Lake Erie and Lake Ontario - interfere with recreational values.

4. They help to purify streams and maintain a favourable oxygen balance, along with other algae.

5. They have been known to foul up fishing nets, e.g. Cladophora - Bay of Quinte.

Examples of Filamentous Green Algae

Unbranched Forms

Spirogyra - This plant is characterized by the presence of spiral chloroplasts which make up a large proportion of the contents of the cell. Pyrenoids, which are centres of starch formation, are arranged along the chloroplasts.

Ulothrix - This algae has a single parietal chloroplast in each cell. The curled edges of the chloroplast are usually evident. Some species have cylindrical cells and others have cells shorter than their width.

Mougeotia - The presence of a plate-like axial chloroplast signifies members of this genus. The chloroplast sometimes shifts its position so that it appears as a narrow ribbon.

Zygnema - A pair of star-shaped chloroplasts signify members of this genus. A large central pyrenoid is always present in each of the chloroplasts. Application of an iodine solution facilitates observation of the chloroplasts.

Oedogonium - Cells of plants of this genus tend to be swollen at the anterior end and replicate end walls are present. Specialized reproductive cells such as zygospores are often visible.

Branched Forms

Cladophora - This genus is characterized by a spreading branching effect with its cells generally cylindrical in shape. The ends of filaments taper very abruptly, with only the terminal cell being involved.

Stigeoclonium - Resembles Cladophora somewhat but tapering off of filaments is more gradual, involving two or more cells.

Chaetophora - Resembels Stigeoclonium but is surrounded by a gelatinous matrix.

Specialized Forms

Chara - Large macroscopic plants growing erect with stem-like branches arranged in whorls and bearing forked leaves. Plants usually feel rough because lime is encrusted on them.

Hydrodictyon - Cells of this alga form a network and it is commonly called "water net" on this account.

B. Non-Motile Green Algae - Coccoid

The coccoid green algae are those that exist as free-floating planktonic units.

Characteristics

1. Since they contain a good deal of starch, a deep purple colour will be produced when treated with iodine solution.

2. Cells of some species often link together to form coenobes. A coenobe is a colony of cells which does not multiply during the life of the colony although the cells do increase in size.

3. There is a great variety in size and shape among the members of this group - cells or colonies may be round, irregular and often ornate.

Significance of Coccoid Green Algae

1. They may cause water to be odourous and may contribute to filter clogging.

2. They may assist in maintaining a favourable dissolved oxygen balance in the water.

3. They are important items in aquatic food chains.

Examples of Coccoid Green Algae

Chlorella

- small cells; generally spherical in shape.
- a single parietal chloroplast is present.

Scenedesmus

- usually 9 to 8 cells - coenobial.
- 4 to 8 cells in a flat plate - usually of cells with rounded ends.

Pediastrum

- flattened colonies of cells often having high numbers of cells in each colony (coenobial).
- wheel-like structure of the colony indicative.

Cosmarium

- are almost as wide as long and have a deep constriction across the centre of the cell called the "isthmus" - forming two semicells.

Closterium

- small in size, no median constriction, cells taper somewhat, but are not sharply pointed.

Ankistrodesmus

- long, slender cells, tapering to a sharp point at each end.
- they may be straight, curved or twisted.

Schroedaria

- solitary, free-floating, often having an "S" shape and pointed at the ends.
- spines may be present protruding from the ends of the cells.

Selenastrum

- cells bent so that tips approach each other, small in size.

Kirchneriella

- resembles Selenastrum but cells are much broader and bent into a definite "C" shape.

LABORATORY - NON-MOTILE GREEN ALGAE

1. Using the key in "Algae in Water Supplies", attempt to identify the alga provided by the instructor.

2. Draw a typical cell of Spirogyra using 450X, labelling all of the parts which make up the protoplasmic content of the cell.

3. Examine the samples of preserved green algae which are available, making rough sketches showing details of their structure for future reference. Sketch at least two coenobial types of green algae.

Flagellates

flagella
nucleus
chlorophyll
eyespot
- cyst

1) Euglenophyta

- eye spot
- single cell

a) - Euglena  - thin membrane
- paramylum insoluble starch reserve

b) - Phacus
- thick cell wall

c) Tracheteromas 
Test or shell
- reproduce by longitudinal fission

2) Chlorophyta (2 or more flagella)

- eyespot near origin of flagella
- unicellular or colonial - starch - pyrenoids

a) Chlamydomonas

1 Contractile
for 4 flagella

c) Volvox

- move in rotating motion

3) Phyrrophyta


- cellulose
- yellow brown
- Cingulum


groove

4) Chyrosphyta

- golden brown - chromatophores
- no starch or pyrenoids - oils present

Synura
- 2 flagella

Dinobryon
- branched tree appearance


Ochromonas
- chromatophore

FLAGELLATED ALGAE

Characteristics

1. Possess flagella - one or more per cell. A flagellum is a whip-like appendage which acts like a propellor.

2. Some flagellates are unicellular, others are colonies of cells held together in gelatinous envelopes.

3. One or more chloroplasts are present which contain chlorophyll.

4. Thick-walled resting stages may be assumed by certain species if unfavourable environmental conditions are encountered.

Significance

1. Many pigmented flagellates can produce strong tastes and odours when they are present in water supplies.

2. Certain pigmented flagellates are able to withstand polluted conditions - tend to indicate polluted conditions if other types which will not tolerate pollution are not present.

3. Filter-clogging results from large numbers of certain flagellates.

4. Release oxygen to the water and utilize carbon dioxide.

Examples of Flagellated Algae

Euglena

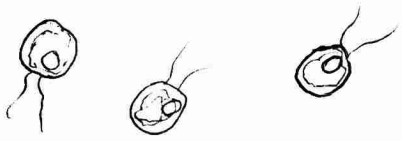
- elongate cells which may be cylindrical or spindle-shaped with a single flagellum - round forms occasionally observed.
- food stored as paramylum (an insoluble carbohydrate) in numerous rod-shaped bodies.
- numerous disc-shaped chloroplasts.
- red eyespot is present which is sensitive to variations in light intensity.
- sometimes become red in colour because of presence of the pigment haematochrome - may cause entire surfaces of ponds to be covered by a bright red film.

Chlamydomonas

- cells usually round or oval with two flagella.
- cells often enclosed in gelatinous sheaths.
- single cup-shaped chloroplast present.
- often found in oxidation ponds and polluted waters.

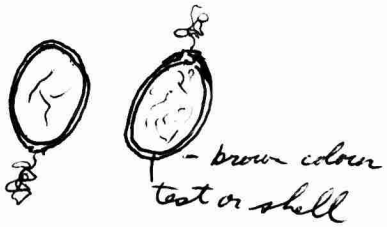
Euglena

C. Chlamydomonas



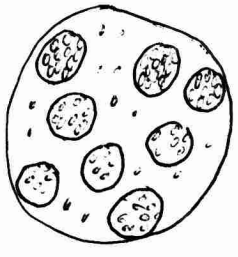
- pear shaped, round or oval
- 2 flagella
- eyespot

Trachelomonas



Volvox

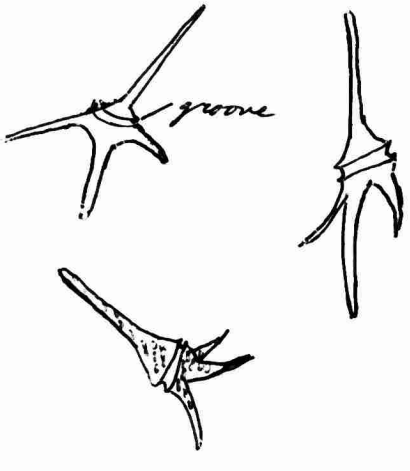
- colonial hollow sphere
- egg-shaped biflagellated
- daughter colonial cells



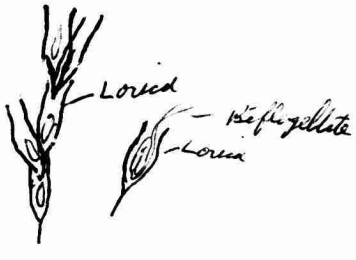
& or & individual cells

Gonium

Ceratium (Armoured Flagellates)



Dinobryon



Carteria

- closely resembles Chlamydomonas but has four flagella instead of two.

Phacus

- cells flattened as seen from the side and often twisted.
- one or more doughnut-like rings of paramylum present.
- long or short tail-piece is present.
- ridges on the cell wall often noticeable.

Trachelomonas

- protoplasm is enclosed in a brown shell called a lorica or test which may be oval or flask-shaped and which has a hole or collar through which a single flagellum protrudes.

Ceratium

- one of the dinoflagellates (armored flagellates).
- brown in colour.
- cell has a transverse groove and one long anterior horn and two or three posterior horns - very distinctive form.
- two flagella - one lies in transverse groove.

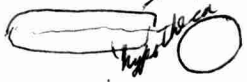
LABORATORY - PIGMENTED FLAGELLATES

1. Examine some of the living flagellates in the samples provided, noting their corkscrew-like movement. Note how Euglena is able to change shape.

2. Follow through the key to identify Euglena. Make a sketch of this organism under medium power, slowing down the organisms with methocel so that the principal parts may be noted and sketched.

3. Examine the various cultures of the flagellates which are available and make sketches to demonstrate the features by which they may be identified.

Diatoms

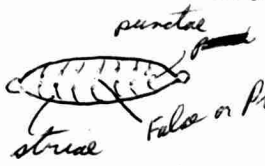
- silica cell walls
- plastids in the cell
- chromatophores - chromatin
- frustules → 

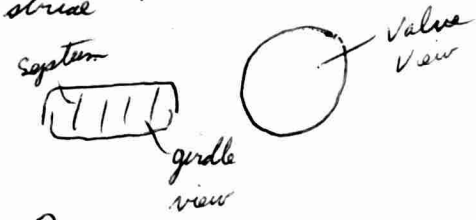
- Cyclotella

Centric Diatom

- Pennate  - long axis
- fission + gamete production

true raphe - channel
- protoplasmic streaming permits movement






- Diatoms

- cause filter-clogging
- taste + odour

Asterionella - star-shaped
- fish odour

DIATOMS

Characteristics

1. Have rigid cell walls made of silica (glass). The cells contain chromatophores which have a brown pigment in addition to chlorophyll. The individual cells or cell walls are called frustules.

2. Diatoms may be unicellular, filamentous or colonial.

3. Each cell resembles a pillbox - two separate parts with one overlapping the other. The "valve" view is that of the top or bottom of the box. The "girdle" view is the side view.

4. Cells are "pennate" which are elongated in structure or "centric" which afford one circular view.

5. Diatoms reproduce mainly by cell division although formation of gametes does occur.

6. Cell markings are evident such as the raphe or pseudoraphe which extends longitudinally, and striae or punctae which are lines of pores extending from the raphe or pseudoraphe to the margin. Nodules at the ends of the raphe may also be present and internal shelves called septa are another feature.

7. Some diatoms can move slowly by a process called protoplasmic streaming.

Significance of Diatoms

1. The most important group of algae which causes filter-clogging.

2. Can produce tastes and odours in water.

3. Water quality can be evaluated by specialists who understand the effects of polluted conditions on numbers of different diatoms.

4. Release oxygen to water, as other algae.

Examples of Diatoms

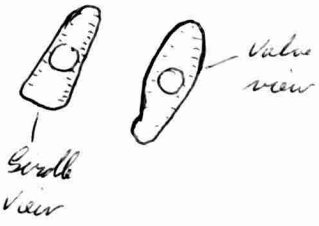
Pennate Diatoms

Synedra

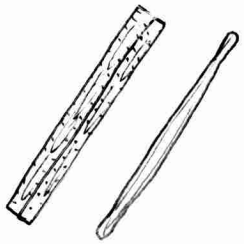
- narrow frustules, much longer than broad - symmetrical.
- may be solitary (most often) or in radially-arranged colonies.
- ends of frustule sometimes pointed, swollen or blunt.
- pseudoraphe is present.

Diatoms

Gomphonema

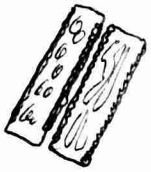


Synedra

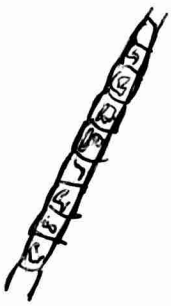


Navicula

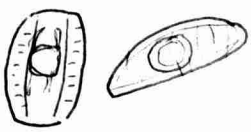
Diatoma



Melosira

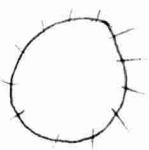


Cymbella



Cocconeis

Stephanodiscus

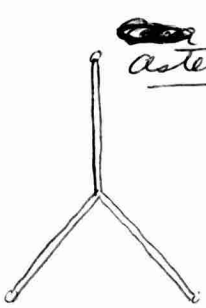


Nitzschia

Actinastrum

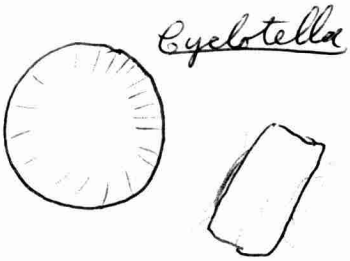
Cyclotella

Asterionella



Fragilaria

Cyclotella



Fragilaria

- linear to fusiform in valve view - symmetrical.
- rectangular in girdle view.
- colonial form with cells in band-like filaments resembling comb-cells joined valve to valve.
- pseudoraphe present.

Tabellaria

- cells united in zigzag chains.
- long cells which are inflated at the centre and with slight inflations at the ends.
- has a narrow pseudoraphe.
- longitudinal septa are present inside the cell.

Navicula

- symmetrical, elongate cells, often boat-shaped.
- has a raphe with central and polar nodules.
- cells are rectangular in girdle view.
- generally single-celled, free floating.

Nitzschia

- elongate cells with varying structure as seen in valve view - may have parallel sides or may be constricted at centre.
- true raphe is present.
- transverse striae or punctae on valve
- generally single-celled.

Asterionella

- cells joined together in star-like colonies.
- inflated ends at centre of star are broader than the free ends.
- cells long, asymmetrical.

Surirella

- elliptical or oval-shaped with rounded ends, asymmetrical.
- solitary cells.
- relatively large size.

Meridion

- Fan-shaped colonies made up of cells joined valve to valve.
- internal septa which show through the wall of the frustule.

Centric Diatoms

Cyclotella

- circular in valve view - smooth region in centre and peripheral lined region (punctae).
- solitary and free floating as a rule.

Stephanodiscus

- rows of punctae extending into centre of cell.
- solitary and free floating.
- spines extend from wall of frustule.

Melosira

- cylindrical cells, sometimes with convex valves and spines which assist in holding cells together in filaments.

LABORATORY - IDENTIFICATION OF DIATOMS

1. Using some of the micro-strainer waste which is available, make up a slide and observe under low power.

2. Note the varied forms which are present, their colour and by pressing lightly on the coverslip, attempt to change the position of individual forms so that different views of the cells may be presented.

3. Note the presence of a true raphe in Navicula and a pseudoraphe (false raphe) in Synedra. The pseudoraphe is formed by interruptions in the lines of dots (punctae) which run transversely across the cell.

4. Make drawings of Stephanodiscus and Navicula under medium power, identifying and naming as many of the structural features as possible.

5. Study and make sketches of as many diatoms as possible, using the reference material available to identify the different genera, with the help of the instructors present.

PLANKTON SAMPLING PROGRAMS

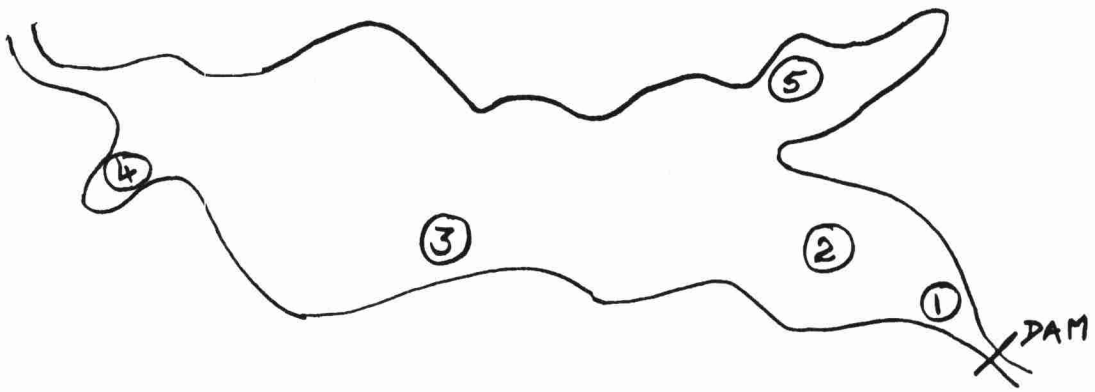
1. An organized program of plankton counting should be based on samples taken at least once a week.

2. Seasonal patterns of algae development tend to repeat themselves year after year and so are relatively predictable - a certain amount of seasonal variation is to be expected, however (variables such as nutrients, rainfall, temperature).

3. More analyses may be desirable if population of a troublesome species increases.

4. It may be easier and less costly to control an upsurge of one particular alga in its early stages.

Location at Sampling Points



1. Both shallow and deep samples should be taken.

2. Samples from top to bottom at one sampling point may be composited to provide a summary for that station.

3. Each major bay or shoal area of a reservoir should be sampled and one should be taken near an intake if the latter is present.

4. Sampling over 24-hour periods or longer may be helpful either studied individually or composited.

Preserving Samples

mercuric chloride solution
36 cc / 40z bottle

1. Samples submitted to the laboratory must be preserved.

2. Formalin at 3 - 5% causes shrinkage of cells and can cause flagellates to explode if these are in the sample - use no more than 3% formalin for any sample.

3. Lugols solution is sometimes used.

4. A .1% merthiolate solution will adequately preserve plankton up to six weeks.

5. A minimum of preservative may be used to render organisms immobile without altering the physiology of the cell too much.

PREPARATION AND ENUMERATION OF PLANKTON

Preparation

1. Whenever possible samples should be studied initially without a preservative being added. Certain of the flagellate forms will either disappear entirely or be severely distorted by formalin as low as 1%. Preservative may be added to kill motile forms for counting after their identity has been established.

2. Unpreserved samples may be refrigerated for future analysis but if they are to be held more than a couple of days, preservative should be added. Commercial formalin is used to make a 3% solution or a more gentle preservative is merthiolate which need only be added to make a .1% solution.

3. Identification and enumeration equipment which is essential includes:

   (1) Compound microscope having:
   - (a) mechanical stage
   - (b) 10X ocular fitted with a Whipple ocular micrometer or reticule which is used to delineate the area to be counted and to measure the areal size of individual cells or pieces of algae.
   - (c) Objectives: approx. 10X
     " 20X
     " 40X
     " 95X

   (2) Sedgwick-Rafter counting cells are essential which measure 50mm x 20mm x 1mm, so that they will hold exactly 1 ml. of sample. Special cover glasses for this counting cell are provided.

   (3) The S-R cell is filled by drawing slightly in excess of 1 ml. of the sample into a pipette after the sample has been well shaken and allowing the sample to flow into one of the openings created by laying a cover glass diagonally across the counting cell. If this is done properly the air will escape from the opening on the opposite side and the cover glass will rotate into proper position by itself to cover the cell. Excess water can be removed by wiping lightly with a soft tissue.

   (4) After filling the S-R cell, it should be allowed to sit on the microscope stage for several minutes to allow the organisms to settle out.

   (5) If many blue-greens are present, iodine may be added to encourage these algae to settle since they tend to float at the surface.

Enumeration

1. Qualitative and Quantitative Analyses

It may only be necessary at times to determine what types of plankton are present and to obtain an approximate idea of their relative numbers. Perhaps a taste and odour problem has developed and it is only essential to determine what particular alga is causing the problem.

However, for analyses to be of lasting value, they must provide a relatively accurate measurement of the numbers of plankton for each genus present. If annual records are maintained, predictions may be ventured since similar conditions tend to develop in cyclic fashion year after year. However, seasonal variations do occur because of factors such as water temperature and amount of sunshine. Information related to water temperature, nature of the weather and cloud cover, water turbidity and pH is all valuable and may lead to more knowledgeable interpretations of what has happened in the past and what may be expected in future. It is only after records have been maintained for several years that accurate forecasting may be a certain accomplishment.

Regular quantitative counts should be made weekly at least. This should provide adequate forewarning of the increase of one particular alga to nuisance proportions so that remedial measures may be implemented before the nuisance condition is an established fact.

The Nature of the Quantitative Count - The Areal Standard Unit Method

The "clump count" has been used in the past but the major disadvantage of this simpler method is that filaments and colonies are counted as units, equal to individual cells. No cognizance is taken of the relative masses of the various organisms which are present.

Procedures related to the areal standard unit method which we will use are as follows:

(1) A Sedgwick-Rafter cell is filled with 1 ml. of the sample. The sample may or may not have to be concentrated depending on the density of organisms in the sample. Concentration is usually necessary. Procedures for concentrating are outlined in the next section.

(2) The areal values of algae in one or two strips across the cell are recorded, or perhaps the areal values for organisms present in a predetermined number of fields (for a concentrated sample) and using appropriate multiplier factors these areal values are projected to areal standard units per ml., litre, etc. An areal standard unit is 400 square microns.

(3) The area which needs to be examined varies with the concentration of algae in the sample. Generally, ten fields are enumerated in a concentrated sample. If the sample is not concentrated because of high numbers of algae, one or two strips are covered.

(4) Half of the cell should be checked using low power to determine whether rotifers or other animal plankton is present in the sample. In this "survey count" the actual numbers of organisms are recorded instead of their areal values.

(5) Before areal standard unit counts can be attempted, the microscope must be calibrated so that the linear and areal values of the lines and squares associated with the ocular micrometer are known (outlined in Section 16 - Calibration).

(6) Before starting the count the sample should be scanned to determine what organisms are present and to establish average areal values for types of algae which are abundant and relatively consistent in size. It may be necessary to use the medium power objective to determine the areal value

of individual organisms more accurately. If this is the case, an ordinary slide must be used and it might be necessary to use the Sedgwick-Rafter concentrating funnel to obtain a sufficiently concentrated sample for this purpose.

(7) Records should be kept of the average areal values of individual species to avoid needless repetition of preliminary measuring. Different species of the same genera may be recorded as Species A, B, C, etc., with their distinguishing features being noted.

(8) For the first while it will be necessary to measure each cell, filament or colony which is encountered to determine its areal value. The individual areal values assigned for each genus are recorded on the bench sheet. When the count is completed, the total areal value for each genus is obtained, and is multiplied by the appropriate factor to obtain the number of areal standard units/ml.

CONCENTRATION TECHNIQUE FOR ALGAE ENUMERATION

Due to several factors such as too few organisms per field, occasional large organisms in isolated fields, clumping, etc, unsatisfactory algae counts can be obtained. To increase the accuracy of algae counts, it is essential to employ a concentration technique by which a large sample volume is reduced to a few milliliters. It is then possible to enumerate organisms in a relatively small number of fields rather than covering an entire strip or perhaps half the cell volume. An appropriate concentration factor must be inserted in the formula to calculate the quantity of algae present per ml of sample.

Methods and materials

A 500-ml Sedgwick-Rafter funnel is required for the concentration. The bottom of the funnel has a one-holed rubber stopper fitted with a glass U-tube which is connected to a suction apparatus by rubber tubing. Silk bolting cloth discs, 3/8 of an inch in diameter, are held tightly in place above the stopper and Berkshire or white sand is added to a depth of 1/2-inch to serve as a filtering medium. Add 5-10 ml of distilled water to wet the sand and drive the air out. Agitate the sample gently and add 250-1,000 ml to the funnel depending on the density of the microscopic organism in the sample. Pour slowly into the funnel, taking care not to disturb the sand. Use slight to moderate suction. Wash down sides occasionally with filtrate or distilled water. Concentrate the sample down to the desired level (usually 5 ml) and then quickly remove the rubber tubing from the U-tube. Remove the rubber stopper allowing the concentrated sample and sand to run into a small beaker. Rinse the funnel and end of the stopper with 20 ml of distilled water <u>over</u> the beaker.

Calculation

If a 1,000 ml sample is reduced to 5 ml + 20 ml of distilled wash water, the concentration factor is $\frac{1000}{25} = 40$.

One ml of the concentrated sample is placed in a Sedgwick-Rafter cell for enumeration. Five to ten fields are counted depending on the density of organisms. It is considered that a degree of concentration that will provide no less than 20-30 organisms per field is essential to ensure reasonable statistical accuracy.

In determining the total count, the following formula is used:

$$\frac{\text{total square microns} \times \text{factor (varies with fields counted)}}{400 \times \text{concentration factor}}$$

The concentration technique should not be used if the sample contains high levels of algae (especially filamentous forms which tend to clump) or large quantities of organic debris.

CALIBRATION OF PLANKTON COUNTING EQUIPMENT

A Whipple Plankton Counting grid must be used in microscopes to delineate the width of counting strips and for measuring areal value of individual organisms. Since the optics of no two microscopes are exactly the same, it is necessary to "calibrate" each instrument against a known scale to determine the linear values of the lines or areal values of the squares making up the counting grid. The Whipple eyepiece must be calibrated for each magnification that is to be used. Microscopes having an adjustable tube length can be set so that the square which is part of the grid covers an area of one square mm. on the counting cell. This occurs when the tube length is 160 mm. However, most of the newer models of microscopes do not have an adjustable tube length.

A. Procedures Involved In Calibration

(1) Installation

To install the ocular micrometer in the eyepiece, carefully unscrew the upper lens and insert the disc, allowing it to slide down until it comes to rest on the shelf inside. Replace the lens and look into it. If the markings on the counting grid are not in sharp focus, remove the disc and turn it over.

(2) Using the Stage Micrometer

The stage micrometer is actually a tiny ruler which is placed on the stage to measure the dimensions of the lines making up the counting grid. The lengths of the various segments of the lines making up the grid should be determined and recorded on the Calibration Data sheet which is present in this section. This must be repeated for each combination of lenses employed.

(3) Lenses Normally Used in Counting Plankton

The 10X eyepiece and the 20X objective are normally used in counting plankton, to provide a total magnification of 200X. The 40X or 45X objective cannot be used with the S-R cell because of the short working distance beneath these medium power objectives.

B. Calculating Factors to Convert to Areal Counts Per Ml

(1) Strip Counts

The S-R cell is 50 mm. long X 20 mm. wide by 1 mm. deep. The total volume of the cell is therefore 1000 mm^3 or 1 ml.

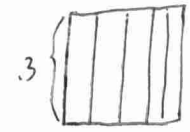
Factor for one strip - strip delineated by vertical lines in the counting grid. One strip the length of the cell in which the distance between the endsof the vertical lines making up the counting grid has been determined to be .5 mm. (or 500 microns) would have the following volume:

$$\begin{aligned} \text{Volume} &= \text{length X width X depth} \\ &= 50 \times .5 \times 1 \\ &= 25 \text{ mm.}^3 \end{aligned}$$

It is necessary to multiply the total count for each genus observed in one strip by a factor obtained by dividing the volume of the strip into the total volume of the cell.

Procedure Field Count (Concentrated)

Formula: Areal Std. Units = $\dfrac{10(\text{no. of fields}) \times .123 \times \text{no. of sq. microns (genus)}}{400 \times 40 \text{ (Conc. Factor)}}$ ($.35 \times .35$)



Volume = $.3 \times .3 \times 1 = .09$ cu. mm.

of 10 fields = $.09 \times 10 = .9$ cu. mm.

Factor = $\dfrac{1000}{.9} = 1111$

Count for each genus = $\dfrac{\text{total sq. } \mu \times 1111}{400 \text{ sq } \mu}$ = areal standard units

- include dilution factor

= $\dfrac{\text{Total sq. } \mu \times 1111}{400 \times 40}$

Areal Std Units $\dfrac{105 \times 14 \times 31.8}{400}$

Using our example, the Factor for 1 strip = $\frac{1000 \text{ mm.}^3}{25 \text{ mm.}^3} = 40$

If two strips are counted, then of course the multiplier factor would be cut in half i.e. 20.

(2) Field Counts

If high numbers of algae are present in a sample it might not be necessary to count one full strip, although this is likely to be the exception rather than the rule.

When high numbers are encountered, ten fields may be examined and each field is delimited by the four lines making up a true square in the ocular grid. The basic relationship previously described still applies, as follows:

$$\text{Factor} = \frac{\text{volume of cell in mm.}^3}{\text{volume examined in mm.}^3}$$

The total volume of the area examined using 10 fields is determined by multiplying the total area of the 10 fields by the depth. Therefore, when the sides of the true square (i.e. one field) in the ocular grid represent a length of .3 mm. (or 300 microns) the volume for 10 fields is as follows:

$$\text{Volume} = (\text{side of Whipple field})^2 \times \text{depth (1 mm.)} \times (\text{no. of fields counted})$$
$$= .3 \times .3 \times 1 \times 10$$
$$= .9 \text{ mm.}^3$$

Therefore the Factor for 10 fields $= \frac{\text{Volume of cell in mm.}^3}{\text{Volume examined in mm.}^3}$

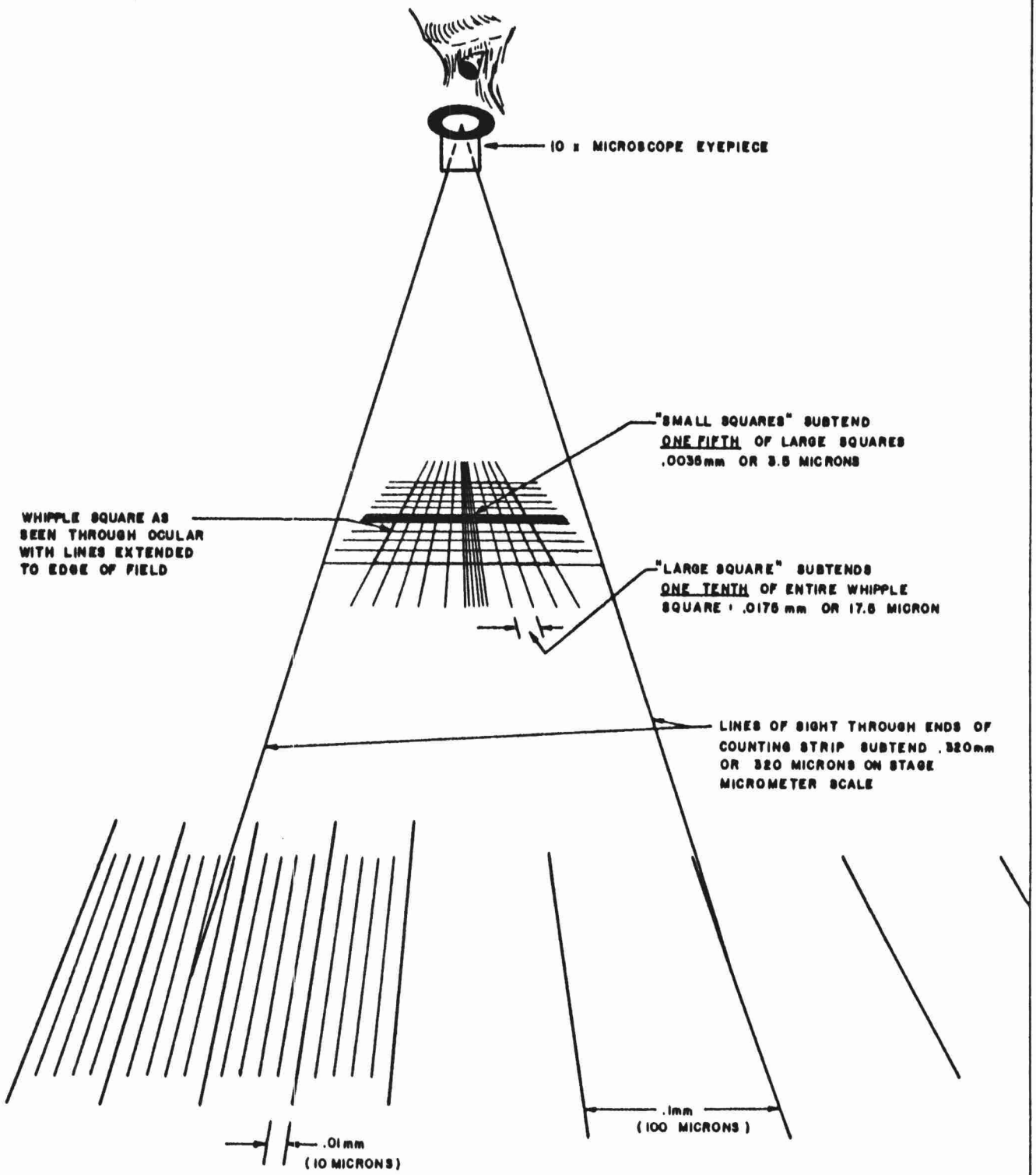
$$= \frac{1000 \text{ mm.}^3}{.9 \text{ mm.}^3}$$

$$= 1111$$

If 20 fields were counted, the factor would then become 555.

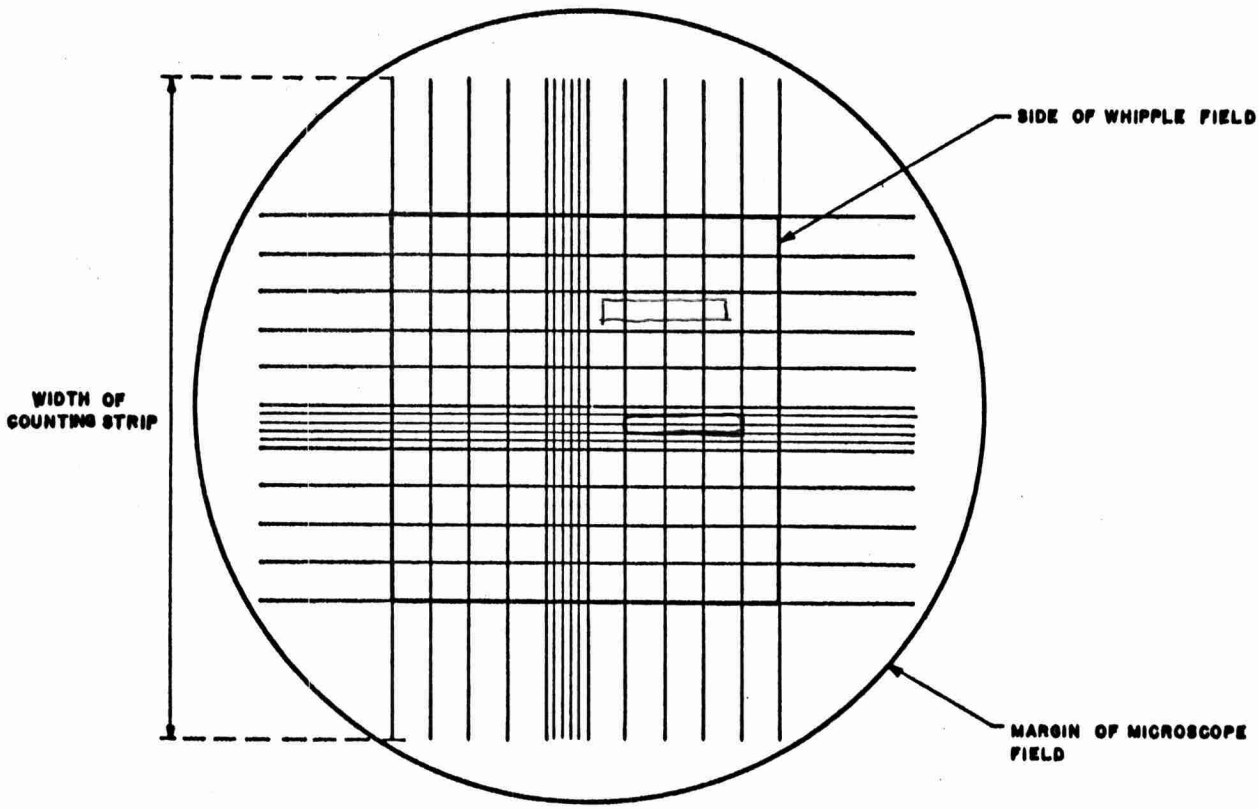
DIAGRAMATIC CALIBRATION OF WHIPPLE SQUARE FOR OWRC MICROSCOPE No. 1130

AS SEEN WITH 10x OCULAR AND 40x OBJECTIVE
(APPROXIMATLY 400x TOTAL MAGNIFICATION)



PORTION OF MAGNIFIED IMAGE OF STAGE MICROMETER SCALE

WHIPPLE COUNTING GRID
AS SEEN THROUGH MICROSCOPE EYEPIECE



MICROSCOPE CALIBRATION DATA

* 1MM = 1000 MICRONS _____ Microscope No. _____

		Linear Dimensions of Whipple Square in Millemeters *			Width of Entire Field	Factor for Conversion to Count/ML.
	OBJECTIVE	WHOLE	LARGE	SMALL		(1 S-R STRIP)
Ocular						
Ocular						

LABORATORY: QUALITATIVE EXAMINATION OF MIXED PLANKTON

1. Using the sample of raw water from your own treatment plant, study and identify the algae present and attempt to list them under the four major groups of algae which have been previously studied in detail. Use the texts available to help in your identifications and solicit the assistance of the instructors present.

2. If you would like additional practice with any one of the major groups ask the instructor to provide the necessary cultures.

LABORATORY: PLANKTON ENUMERATION

Using your own microscope, if you have one fitted with a suitable ocular micrometer, undertake a plankton count in one strip across the S-R cell, using the areal standard method. Record your count on a bench sheet.

Check with one of the instructors present to ensure that you are using the proper multiplier factor for your microscope.

Count a single strip using the sample which is available and which has previously been counted in our laboratory. Use a bench sheet and then record your count on a report form.

Continue practising counts using your own water sample or any of the other samples which are available. Compare results of your counts with our records.

Counting Procedures

Shake the sample thoroughly to evenly distribute the plankton in the sample, but not so hard that individual colonies will be disrupted.

Concentrate the sample in accordance with the instructions provided in a previous section.

Transfer 1 ml. of the sample to the S-R cell as described previously. Remove the aliquot from the sample bottle before the organisms have time to settle to the bottom. After the cover glass rotates properly into position, wipe off excess water lightly with a tissue. The cover glass should remain intact on the S-R even when turned upside down.

Set the S-R cell on the microscope stage and allow to stand for several minutes to enable the organisms to settle to the bottom of the counting cell.

Select ten randomly distributed fields for the count.

In a concentrated count, include all of the organisms lying within the Whipple field. (see p. 16-4).

All of the organisms lying between the ends of the vertical lines in the ocular grid should be included in the count. Organisms which lie across the ends of these lines should be counted at the top, but not at the bottom.

For each field counted, identify and measure the individual cells and colonies as they are encountered by means of the lines and squares making up the ocular grid. The fine adjustment knob should be turned so that you can determine whether all of the organisms in each field are resting on the bottom. Any other organism observed at various levels must be included in the count. Determine the areal value for each cell or colony as they are observed and record on the bench sheet.

When your tally is completed for the ten fields or entire strip, multiply the total for each genus by the required multiplier factor to give you the total areal value for each in the entire ml. in the counting cell. Divide the total for each genus by 400 to obtain the number of areal standard units of each genus per ml. In field counts, when the sample has been concentrated, the total must be further divided by the approximate concentration factor.

A "survey count" should be made quickly using low power over ½ of the counting cell to determine the numbers of animal plankton present. These are recorded by number rather than by assigning areal value.

LABORATORY: SPECIALIZED STUDIES RELATED TO ENUMERATION

Your microscope has been centred on one particular cell, colony or filament of algae. Assign what you believe to be the correct areal value to your organism and record the areal value and the number of the microscope. Return the organism to its original position when you finish. Go to all of the other microscopes in turn and repeat, recording the areal values and numbers of the microscopes as you proceed.

From the sample which has been provided, do an areal count involving ten fields and calculate the appropriate multiplier factor which you must use to project to areal standard units per ml.

PLANKTON REFERENCES

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   By: G. W. Prescott
   Published by Cranbrook Institute of Science - Bulletin Nos.31 1951
   - nothing on desmids and diatoms
   - approximate cost $10.50 - $12.00

2. Fresh-Water Biology - Second Edition
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   edited by W. T. Edmondson
   Published by John Wiley & Sons, Inc., New York
   - algae and animal plankton as well.

3. How to know the Fresh-Water Algae
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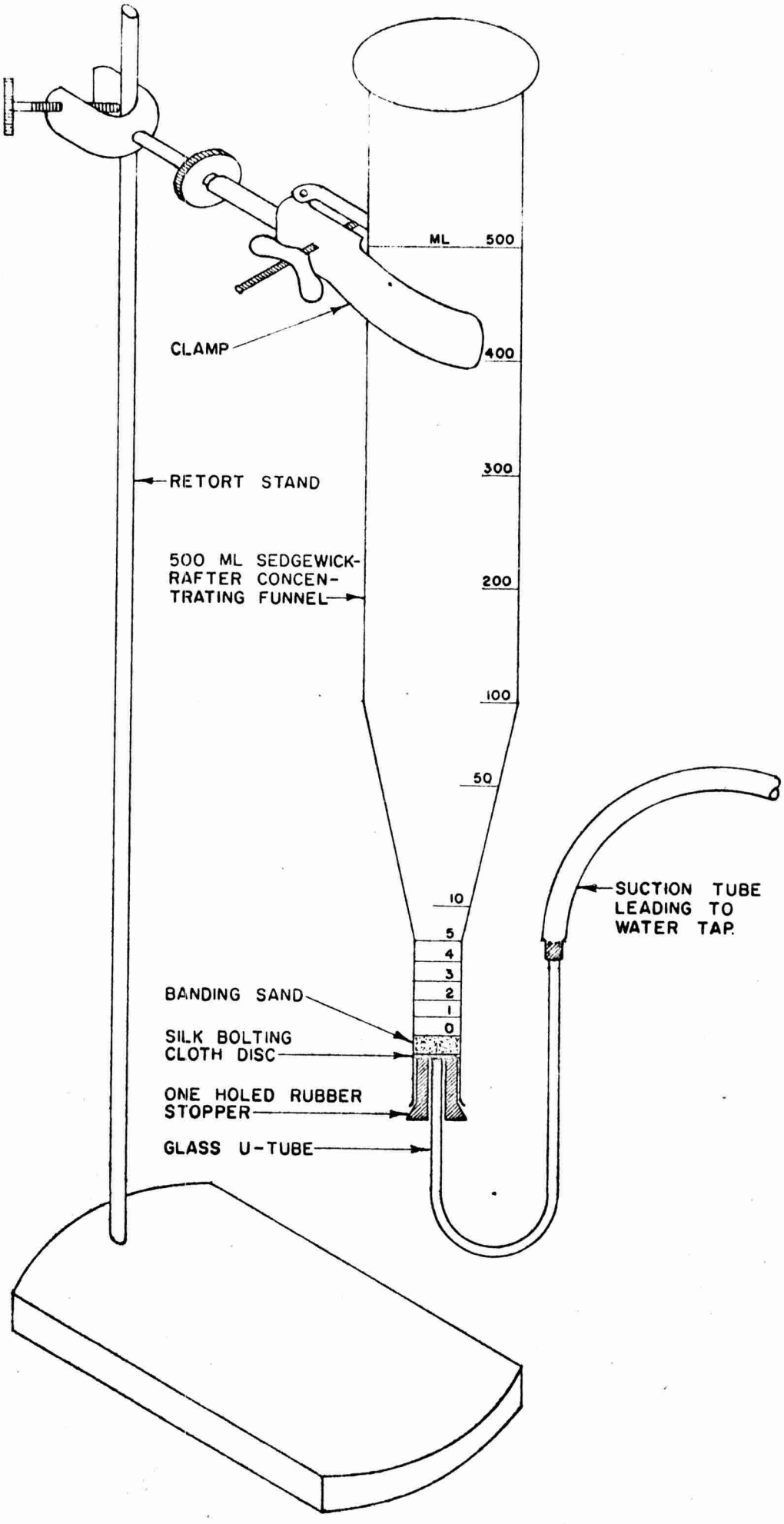
   Michigan State University Press Lansing, Michigan

9. Handbook of Algae - 1954
   By: H. S. Forest
   The University of Tennessee, Knoxville, Tennessee.

EQUIPMENT FOR CONCENTRATING ALGAE



BENCH SHEET - PLANKTON ENUMERATION

Sampling Point (Raw Water etc)_____ Date Sampled_____ Sample No._____
Date Analysed _____ by_____ Factor: Algae _____
Enumeration Procedured Used:_____ Zooplankton _____

Algae		Total Square Microns	Areal Standard Units per ml (total x factor / 400)

Total Areal Standard Units/ml

Zooplankton		Total No.	Total No/ml

Total No. Zooplankton/ml

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